

ORIGINAL ARTICLE

Evaluation of Procalcitonin Accuracy for the Distinction Between Gram-Negative and Gram-Positive Bacterial Sepsis in Burn Patients

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Sepsis is the main cause of death in burns. Early institution of antimicrobial therapy is crucial to optimize outcomes but superfluous therapy increases adverse events, microbial resistance, and costs. Blood cultures are the gold standard for diagnosis but can take 48 to 72 hours. Biomarkers are used to help sepsis diagnosis and distinction between Gram-negative and Gram-positive bacterial cause. The aim of this work is to evaluate procalcitonin (PCT) accuracy for this distinction in burn patients. Retrospective observational study of adult septic burn patients with $\geq 15\%$ total burn surface area admitted from January 2011 to December 2014 at a Burn Unit in Portugal. A statistical analysis was done, evaluating the correlation between PCT levels on the day of the first positive blood culture and microbiological data for Gram-negative and Gram-positive bacteria. Patients with mixed bacterial and/or fungal blood cultures were excluded. Data were summarized by quartiles statistics. Blood cultures were positive in 189 patients: 75 (39.7%) showed growth for Gram-negative and 114 (60.3%) for Gram-positive bacteria. Patients with Gram-negative bacteria have significantly higher PCT levels. Receiver operating characteristic curve analysis showed accuracy for Gram-negative discrimination with area under the curve = 0.687. Most elevated levels were related to nonfermentative Gram-negative bacteria and by *Klebsiella pneumoniae* and other Enterobacteriaceae. PCT levels were significantly higher in burn patients with Gram-negative sepsis comparing to patients with Gram-positive sepsis and controls. The determination of PCT levels may help the choice of empirical antimicrobial therapy while microbiological culture results are not available, despite not fully ensuring the desirable degree of precision.

An early and adequate antimicrobial therapy is the main step for the management of septic patients.¹ Unfortunately, differential diagnosis between sepsis and the systemic inflammatory response triggered by trauma is difficult, particularly in burn patients where the usual clinical signs of sepsis are frequently present even in the absence of microbial infection.² For instance, burn injuries leading to upregulation

of the hypothalamic thermal center, physiologic release of catecholamines and cytokines, shift of fluids and the consequent cardiovascular changes, can produce hyperthermia, tachycardia, hypotension, etc., that are transitory and do not reflect any microbial invasion but just a tentative of adjustment of human body systems to the changes in the homeostatic equilibrium.

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Authors' Contributions. All authors have read the manuscript and agreed to its content, are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria and gave their consent for publication. L.C., V.A., C.C., and M.C. designed the study, interpreted data, and draft the manuscript. V.A. was responsible for most of statistical analysis. R.M., M.V., and J.G.F. were responsible for data acquisition, search of literature, and made suggestions for its integration along the manuscript. C.C. made also substantial intellectual contributions for the Introduction and Discussion sections of the manuscript. L.A. and J.A.P. review the manuscript, and made useful suggestions for Discussion and Conclusions sections.

Ethics Approval and Consent to Participate. Being a retrospective observational study of patients from an anonymized dataset, involving only recording data from the medical record, the Ethics Committee from Coimbra University Hospital Centre (CHUC), waived the need of informed consent according to Declaration of Helsinki and CIOMS International Ethics Guidelines.

Consent for Publication. As all data was anonymized, this study does not contain any individual person's data in any form (including individual details, images, or videos) and accordingly consent for publication was waived.

Availability of Data and Material. The data that support the findings of this study are available from the datasets of the Informatics Department of Coimbra University Hospital Centre but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors, upon reasonable request and after permission of the Ethics Committee from Coimbra University Hospital Centre.

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The gold standard for sepsis diagnosis still relies on the microbiological growth in blood cultures,³ which can take as long as 48 to 72 hours, according to different facilities,⁴ and the antimicrobial sensibility tests may be available even later. On the other hand, the adequacy of antimicrobial therapy is obviously related with the appropriateness of the chosen drugs, that is, the selection of the most efficacious drug against the causative microorganism. In practical terms, physicians have to assess the presence of sepsis in a complex clinical setting, with great chance of misdiagnosis (false positive or false negative), and in most cases to wait 2 days to confirm their suspicion, having (or not) prescribed an antimicrobial therapy that may be inefficacious against the causative bug, allowing the septic process to progress and increasing the likelihood of a fatal outcome. Moreover, a superfluous or an inappropriate antimicrobial therapy presents risks of adverse events for the patient and stimulates the development of microbial resistance, besides increasing costs.⁵ In conclusion as described in a seminal work by Kumar et al,⁶ it is of outstanding importance the prompt institution of an effective antimicrobial therapy, avoiding the serious risks present when this is not timely done.

In the last decade, biomarkers have been employed to help sepsis diagnosis and antimicrobial prescription and stopping. Together with infection control measures and antimicrobial therapy protocols, the use of biomarkers constitutes the backbone of most antimicrobial stewardship programs.⁷ From a multitude of clinical and biochemical biomarkers described in literature, procalcitonin (PCT) became one of the most employed due to 1) its relatively good accuracy for the diagnosis of septic and nonseptic patients since the first hours of microbial invasion, helping the decision to start or postpone antimicrobial therapy, particularly if used in a dynamic approach; 2) correlation between PCT levels and sepsis severity, and 3) its rapid fall when infection is controlled.⁸ Furthermore, significant differences in PCT levels have been found according to the causative pathogens, namely between Gram-negative and Gram-positive bacteria, which facilitates the choice of the drugs to be empirically used meanwhile blood culture results and sensibility tests are not available.

Even being controversial for some authors, determination of PCT serum levels has been consistently advocated for the diagnosis, prognosis, and antimicrobial stewardship in burn patients. Taking into account the different therapeutic approach to different pathogens, it is worthwhile to evaluate the discriminative potential of PCT to set the more appropriate empirical therapy. The aim of this work is to size up PCT performance for the differential diagnosis between sepsis by Gram-negative and Gram-positive bacteria in a large sample of burn patients.

MATERIALS AND METHODS

Patient Informed Consent

Considering that this was an observational study using anonymized retrospective data, the Independent Ethics Committee (Comissão de Ética para a Saúde, Coimbra Hospital University Center—CHUC, Coimbra, Portugal) waived the need of informed consent.

Study Plan

Data for this retrospective observational study was collected from the clinical files and laboratory electronic records of consecutive burn patients with 15% or more of total burn surface area, admitted from January 2011 to December 2014 at Coimbra Burn Unit (CBU), a department of CHUC. All the patients had positive blood cultures and clinical diagnosis of sepsis, following the American Burn Association criteria⁹: suspicion of infection coupled with the presence of three or more of the following parameters: temperature $>39^{\circ}\text{C}$ or $<36.5^{\circ}\text{C}$; tachycardia >110 beats/min; tachypnea >25 breaths/min or ventilation >12 l/min; thrombocytopenia $<100,000/\text{ml}$; hyperglycemia (untreated plasma glucose >200 mg/dl or intravenous glucose requirement >7 U/h over 24 hours; enteral feeding intolerance: abdominal distension or gastric residuals more than two times feeding rate or diarrhea >2500 ml.

Blood cultures were obtained in a standardized way. Three samples were collected by sterile venepuncture in septic patients. Except when immediate antimicrobial therapy has to be initiated due to sound clinical or laboratorial sepsis suspicion, the collects were done in the morning (7–8 am). This collect was repeated every 2 days until clinical resolution and PCT normalization.

Using sample patients who never developed sepsis during its stay at CBU as controls, a statistical analysis was done to evaluate possible correlation of PCT levels on the day of the collection of the first positive blood culture with microbiological data, according to two groups of microorganisms: Gram-negative and Gram-positive bacteria. To avoid potential bias and simplify the analysis, patients with positive mixed bacterial and/or fungal blood cultures were excluded from the study. When a patient had more different microorganisms present in the blood cultures at different timepoints, only PCT levels of the first identification were subjected to analysis. If a patient had more than a PCT measurement on the day of collection, the highest value was used for the analysis. PCT was measured with TRACE© (time-resolved amplified cryptate emission) technology (Kryptor© PCT; Brahms© AG; Hennigsdorf, Germany).

Statistical Analysis

Data were summarized by quartiles statistics. The quantitative variables under study showed a non-Gaussian distribution and thus a nonparametric approach (Kruskal–Wallis and Mann–Whitney tests) was used to compare quantitative variables. Qualitative variables were compared with the Pearson chi-square test. For pairwise comparisons, the Bonferroni correction was applied.

Receiver operating characteristic (ROC) curves, in particular the area under the curve (AUC), were performed to evaluate PCT ability in Gram-negative and Gram-positive discrimination. Sensitivity, specificity, positive and negative predictive values were calculated for some cutoff values including the best cutoff defined by the maximum value of Youden index ($J = \text{sensitivity} + \text{specificity} - 1$).

Statistical analysis was performed with SPSS© 25.0 IBM© for Windows©. A p value of less than .05 was set as the level of significance and the confidence intervals are reported with 95% confidence level.

RESULTS

The sample under analysis was composed 438 burn patients. Among these patients, 249 (56.8%) did not fulfill American Burn Association sepsis criteria neither had any growth in their blood cultures during their stay at CBU, being deemed to serve as controls. Blood cultures were positive in 189 (43.2%) patients; among from these, 75 patients (39.7%) showed growth for Gram-negative bacteria and 114 (60.3%) showed growth for Gram-positive bacteria (Table 1). The median age was 62 years for controls, 66 years for patients with sepsis by Gram-negative bacteria and 69 years for patients with sepsis by Gram-positive bacteria; the difference among groups did not reach statistical significance. The same was true for gender distribution, which showed a preponderance of the masculine sex: control patients included 152 males (61%) and 97 females (49%); the Gram-negative group was composed by 41 males (55%) and 34 females (45%) meanwhile the Gram-positive group gathered 70 males (61%) and 44 females (39%; Table 1).

On the day of the first identification of microbiological growth in blood cultures, PCT levels were significantly higher in patients with Gram-negative bacteria comparing to controls and patients with Gram-positive bacteria; the differences between controls and Gram-positive infected patients did not reach statistical significance (Table 2).

Figure 1 depicts box-plots for PCT levels in the first day of microbiological identification, clearly showing higher values for patients in the Gram-negative group in relation to control group and to Gram-positive group while the difference between these later groups is not evident.

The maximum value of the Youden index was 0.31, for a cutoff = 0.57 ng/ml. This cutoff reached a sensibility of 63% and a specificity of 68%; the corresponding positive predictive value was set in 57% and the corresponding negative predictive value achieved 74% (Table 3). This was the optimum PCT cutoff, corresponding to the maximum point of the ROC curve: higher ones were associated with lesser sensitivity and lower ones led to loss of specificity.

ROC curve is presented in Figure 2. The AUC showed a significant accuracy for Gram-negative discrimination from Gram-positive: AUC = 0.687, with 95% confidence interval = 0.609–0.765.

Subgroup analysis was performed including the most frequent Gram-negative and Gram-positive microorganisms responsible for sepsis in this sample of patients. In the Gram-negative group, the mostly frequently isolated

agent was *Pseudomonas aeruginosa*, as it would be expected according to its great prevalence in many burn units, followed by *Acinetobacter* spp. and other nonfermentative bacteria, including *Burkholderia cepacia* and *Stenotrophomonas maltophilia*. The Enterobacteriaceae were also very common, namely *Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus mirabilis*, etc. From the Gram-positive group, *Staphylococcus epidermidis*, *Staphylococcus hominis* and other coagulase-negative species of Staphylococci, some of them without more specific identification furnished by the laboratory, were the most isolated from the blood samples. As it happened with *Bacillus* spp. and *Corynebacterium* spp., most of times the coagulase-negative species of Staphylococci species were suggested to be probable contaminants in the microbiological results and sensibility tests. Group D Enterococci (namely *Enterococcus faecalis* and *Enterococcus faecium*) and *Staphylococcus aureus* were also very frequently isolated and there were also isolations of *Streptococcus* spp. Table 4 displays the list of the most common microorganisms and the corresponding values of PCT levels on the first day of microbiological identification. The full list can be found in Supplementary Annex I.

Despite the presence of several outliers, it was found that PCT levels in the Gram-negative group were in general significantly higher comparing to controls, what did not happen in the Gram-positive group, with the exception of patients with sepsis due to *Streptococcus* spp. (Figure 3). With the exception of those with sepsis due to this Gram-positive species, which isolation is rare at CBU, in almost all patients with PCT concentrations above 3.00 ng/ml on the day of collection of the first positive blood culture, the causative microorganism was a Gram-negative agent.

In the first case, the statistical difference was more pronounced for glucose nonfermenting bacilli (particularly *Acinetobacter*, *Pseudomonas*, and *Burkholderia* spp.) and for *E. coli* and *K. pneumoniae*, glucose fermenting rods from the Enterobacteriaceae family. Among patients with sepsis due to Gram-positive cocci, PCT levels only reach statistically significant difference for *Streptococcus* spp., as referred, but there was a trend for significance for *Enterococcus* spp. and for *S. aureus* (not visible for nonaureus species).

DISCUSSION

The statistical analysis of PCT levels on the first day of microbiological identification in blood samples in this sample

Table 1. Population characteristics

		Controls	Gram-Negative Sepsis	Gram-Positive Sepsis	P
Number of Patients		249	75	114	—
Age (years)	Median	62.0	66.0	69.0	
	Q1–Q3	45.5–78.0	44.5–79.5	47–80.0	.392*
	Males	152 (61%)	41 (55%)	70 (61%)	
Sex	Females	97 (49%)	34 (45%)	44 (39%)	.578†
Procalcitonin (ng/ml)	Median	0.20	0.75	0.32	.000*
	Q1–Q3	0.11–0.84	0.35–4.15	0.16–0.87	

*Kruskal–Wallis test.

†Chi-square test.

Table 2. Pairwise comparisons for procalcitonin levels between sepsis groups

Comparison	P
Gram-negative septic patients vs controls	.000
Gram-negative septic patients vs Gram-positive septic patients	.000
Gram-positive septic patients vs controls	.153

Mann–Whitney test with Bonferroni-corrected *P*-values.

of extensively burned patients confirmed previous reports demonstrating significantly higher values in the presence of Gram-negative bacteria comparing with controls or patients with Gram-positive sepsis.^{10–13} The difference was most pronounced when causative agents were glucose nonfermenting bacilli, particularly *Acinetobacter* and *Pseudomonas* spp., or Enterobacteriaceae rods, like *E. coli* or *K. pneumoniae*. On the other hand, a statistical difference in PCT levels was not found between in PCT levels of patients with sepsis caused by Gram-positive bacteria and control patients, with the exception of patients with sepsis caused by *Streptococcus* spp.

The results of this work are consistent with medical literature. Opal and Cohen¹⁴ attributed the different characteristics of sepsis caused by Gram-negative and Gram-positive to the different constitution of their respective cell membranes, which will trigger different immunological responses and are, in most part, correlated with diverse clinical presentations and outcomes.¹⁵ Briefly explaining, despite there is not yet a full understanding of the mechanisms involved in cytokines activation following microbial insult, it is consensual that human innate immune cells (macrophages, neutrophils, dendritic cells) have receptors, present either on the external cell membrane or inside the cytoplasm (endosomes) which are

apt to recognize specific circulating molecular patterns. These pattern recognition receptors (PRRs) can be activated by molecular patterns resulting from nonmicrobial tissue damage (damage-associated molecular patterns, DAMPs) or by those exclusively corresponding to microbial pathogenic components (pathogen-associated molecular patterns, PAMPs).¹⁶ The interaction between PRRs and PAMPs induces the release of cytokines by immune cells, initiating the septic process.

There are several types of PRRs, including Toll-like receptors (TLRs) and NOD-like receptors—mainly activated by bacteria; RIG-I-like receptors and DNA-sensing molecules—crucial for sensing of viruses; C-type lectin receptors responding to fungi and mycobacteria PAMPs; etc. The outer membrane of Gram-negative bacteria cell wall is composed mostly by lipopolysaccharide, frequently referred as endotoxine, which is its principal PAMP, being recognized by TLR4.¹⁷ Instead of lipopolysaccharide, PAMPs of Gram-positive bacteria cell wall are basically lipoteichoic acid,¹⁸ lipoproteins and proteoglycans, mostly sensed by TLR2.

TLR4 activation triggers a strong release of inflammatory cytokines, namely tumor-necrosis factor α , interleukin-1, and interleukin-6.¹⁹ These cytokines will promote gene transcription leading to PCT secretion from extrathyroidal tissues, with abrupt rise of its blood levels. It was also described a direct stimulation of PCT secretion by circulating endotoxins.²⁰ On the other hand, TLR2 activation usually induces a relatively weaker and not always straightforward production of those cytokines, varying according to different pathogens by not well known reasons.

In 2008, Charles and colleagues analyzed the accuracy of PCT measurements to discriminate between Gram-negative and Gram-positive bacteremia at the onset of bloodstream infection, concluding that serum levels were greater in the

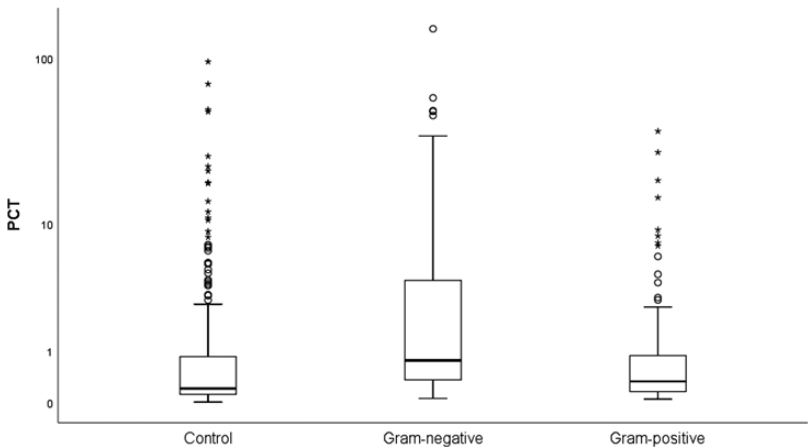


Figure 1. Box-plots for procalcitonin levels in controls (*n* = 249), Gram-negative (*n* = 75), and Gram-positive (*n* = 114) sepsis patients groups.

Table 3. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of procalcitonin cutoffs for the distinction between Gram-negative and Gram-positive sepsis in burn patients

Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden	Obs.
0.50	64	62	53	72	0.26	
0.57	63	68	57	74	0.31	Max. Youden
1.00	46	76	52	71	0.22	
5.00	23	92	62	68	0.15	

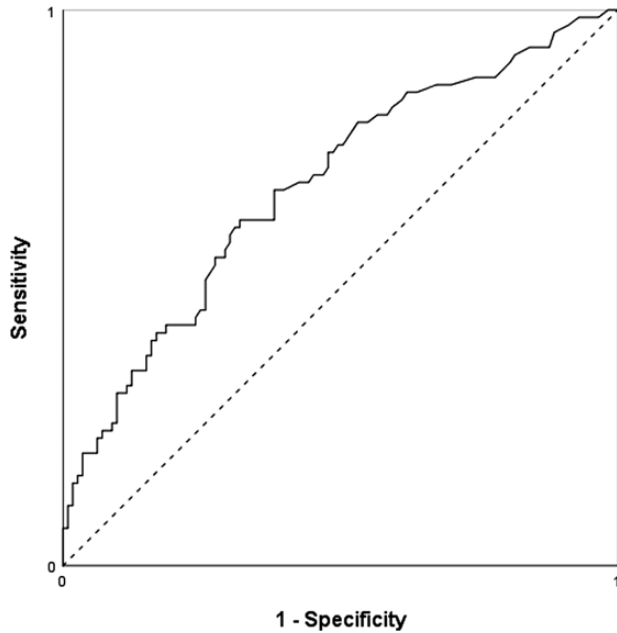


Figure 2. Receiver operating characteristic curve for procalcitonin accuracy in the distinction between Gram-negative and Gram-positive bacterial sepsis in burn patients (cutoff = 0.57 ng/ml).

first group, with an AUC of 0.79, opposing to what happened with the measurements of C-reactive protein and leucocyte counting.²¹ As PCT levels determination is available sooner than Gram stain results and microbiological identification, these authors suggest this information should be taking in account when choosing empirical antibiotherapy for sepsis.

In 2012, Jeong et al,²² showed a good performance of PCT in the distinction between patients with negative and positive blood cultures, facilitating the differentiation of true blood-stream infections from contamination. They also reported that significantly higher values were found for Gram-negative bacteremia comparing to Gram-positive or fungal ones, what

did not happen with C-reactive protein values. With most patients coming from hematological ICUs, an article by Brodská et al²³ described significantly higher PCT values for patients with Gram-negative bacterial infections, comparing to Gram-positive or fungal infections, meanwhile no statistical difference was found between these latter two groups. They concluded that PCT levels could be used to help the confirmation or exclusion of Gram-negative sepsis. Nakajima et al,²⁴ presented similar results in 2014, speculating the possibility of using PCT levels to help antimicrobial empiric antibiotherapy.

In 2015, Oussalah et al²⁵ using a comprehensive electronic database, performed an observational cross-sectional study and analyzing 2699 patients with positive blood cultures, found statistically higher PCT levels in patients with Gram-negative sepsis comparing patients with Gram-positive sepsis, with most elevate values for *Escherichia* spp., *Bacteroides* spp., *Klebsiella* spp. and *Enterobacter* spp. They also pointed values under 0.75 ng/ml as very effective for exclusion of most clinical relevant pathogens, meanwhile a cutoff above 10 ng/ml practically excluded the hypothesis of sample contamination or fungal infection. In a prospective study, including 1949 adult patients with positive blood cultures, Leli et al²⁶ also reported significantly higher PCT levels for Gram-negative infections, more pronounced for Enterobacteriaceae bacteria, suggesting a cutoff of 3.1 ng/ml for the exclusion of these microorganisms. Guo et al²⁷ reached the same results in a sample of 280 septic patients and listed *Klebsiella*, *Escherichia*, *Acinetobacter*, *Enterobacter*, and *Pseudomonas* as the pathogenic species responsible for higher PCT levels. In 2016, Li et al²⁸ analyzing 328 septic episodes, suggested that PCT levels might be used as a surrogate marker to distinguish sepsis cases originated by Gram-negative bacteria from the ones deriving from Gram-positive bacterial or fungal invasion of bloodstream, proposing a cutoff of 2.44 ng/ml. Yan et al²⁹ reviewed data from 484 monomicrobial positive blood cultures of septic patients (75% collected at the ICU and 25% at the Emergency Department), reporting statistically significant differences in PCT levels, with higher values corresponding to patients

Table 4. Procalcitonin values for the most frequently isolated groups of microorganisms in blood samples of septic burn patients

Microorganism	Number	Median	Q1	Q3	P
Controls	249	0.20	0.11	0.84	
Glucose nonfermenting Gram-negative Bacilli					
<i>Acinetobacter</i> spp.	13	1.17	0.49	7.30	.002
<i>Pseudomonas</i> spp.	13	0.67	0.39	1.68	.005
<i>Burkholderia cepacia</i>	4	1.82	0.89	3.05	.045
<i>Xanthomonas maltophilia</i>	4	0.63	0.29	8.89	.241
Enterobacteriaceae					
<i>Enterobacter</i>	9	0.55	0.22	0.62	.087
<i>Escherichia coli</i>	5	2.96	0.75	6.90	.020
<i>Klebsiella pneumoniae</i>	5	1.77	0.58	22.18	.043
<i>Serratia marcescens</i>	5	0.75	0.48	0.89	.255
Gram-positive Cocci					
<i>Enterococcus</i> spp.	12	0.38	0.18	0.73	.177
<i>Staphylococcus aureus</i>	11	0.28	0.21	0.97	.185
<i>Staphylococcus (except aureus)</i>	54	0.29	0.11	0.88	.668
<i>Streptococcus</i> spp.	8	2.18	1.27	4.91	.003

Mann-Whitney test (comparison with control).

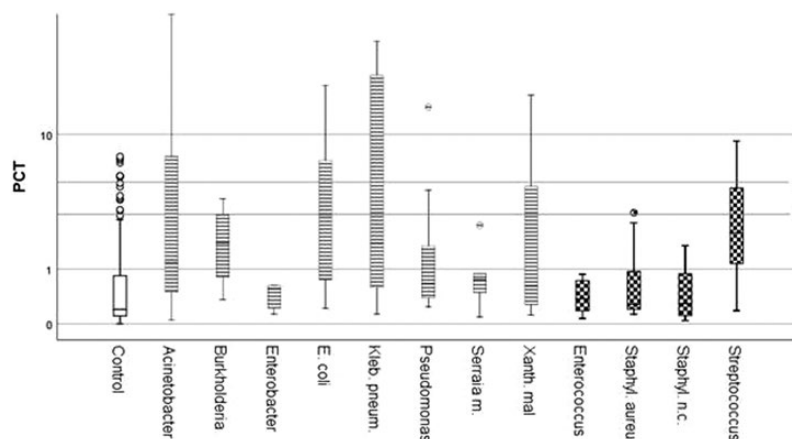


Figure 3. Box-plots for procalcitonin levels in Gram-negative and Gram-positive bacterial sepsis subgroups.

with Gram-negative infection. From the Gram-negative bacterial sepsis group, PCT levels were more pronounced for Enterobacteriaceae microorganisms (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp., by this order), which presented relatively higher values than glucose nonfermenting Gram-negative bacilli (*P. aeruginosa*, *Acinetobacter baumannii*, *S. maltophilia*, *B. cepacia*, etc.). From the Gram-positive bacterial sepsis group, patients infected by *Streptococcus* spp., *Enterococcus* spp., and *S. aureus* had the most elevated PCT concentrations. The authors defended that PCT could be useful not only to distinguish between Gram-negative and Gram-positive sepsis, but might even be employed to identify diverse species inside each of these groups of microorganisms.

In a work from 2018, Thomas-Rüddel et al performed a secondary analysis of a prospectively collected dataset, including a very large sample with 4858 septic patients from 40 hospitals. Their results were very similar to the present study, showing distinctly higher values for PCT concentrations in patients with Gram-negative bacteremia than in patients with sepsis resulting from Gram-positive or fungal systemic invasion.³⁰ Indeed, the AUC for the discrimination of Gram-negative sepsis from Gram-positive was identical, in spite of substantially diverse cut-offs. Subgroups of pathogens with the most elevated values were also very close, with *Streptococcus* spp.; *E. coli*, *Proteus* spp., *K. pneumoniae* and other Enterobacteriaceae on the top. The authors referred, however, a large overlap of PCT levels and speculate that higher values may be more related with higher bacterial load and potentially with intrinsic characteristics of pathogens groups, considering the discriminatory power too low to guide therapeutic decisions.

Burn patients have a risk of infection superior to the average critical care patient and sepsis diagnosis is more difficult³¹ due to the intense inflammatory systemic response unleashed by the burn insult *per se*. In these patients, PCT measurements, particularly using a kinetic approach, have been increasingly advocated by many authors to help the differentiation between pure inflammatory reaction and microbial infection^{32–36} and for antimicrobial stewardship.³⁷ However, this strategy is still not fully accepted^{38–42} in spite of systematic reviews and meta-analysis suggesting its validity.^{43–45} In 2012, a study of Lavrentieva et al,⁴⁶ including 86 burn patients, was presumably the first work reporting statistically significant differences of PCT levels between burn patients with Gram-negative sepsis and those with Gram-positive sepsis, with the

most elevated values in the former group. Mokline et al in a paper of 2015,⁴⁷ including 44 patients, confirmed these results.

To the authors' knowledge, the present work, with 189 septic burn patients, from a homogenous population, corresponds to the largest sample already analyzed in medical literature regarding this subject. It confirms previous reports and, moreover, it further details subgroups differences. On its strengths one can also count the use of strict and internationally validated criteria for definition of burn sepsis, as well as the exclusive utilization of microbiological positive bloodstream cultures, collected in a standardized way, avoiding potential bias due to the use of other types of biological samples. The results of this study, with PCT showing a fair capacity for the distinction between Gram-negative and Gram-positive sepsis insinuate the possibility of using its values in face of sound suspicion of sepsis in burn patients to help the choice of empirical therapy until definitive microbiological identification is available. Cutoffs will be clearly dependent on the idiosyncratic characteristics from each facility, depending on its nosocomial flora and its patients and cannot be generalizable. However very high PCT levels (for instance, above 3.00 or 5.00 ng/ml) would usually be more associated with Gram-negative sepsis, with fair positive predictive value and negative predictive value, and good specificity in spite of outliers may be present. Also, in the great majority of the cases, PCT values under 0.5 ng/ml will not correspond to Gram-negative infections but to Gram-positive or fungal ones.

Paying attention that the majority of deaths in burn patients result from infectious episodes and sepsis⁴⁸ is clear that prompt, adequate and appropriate selection of antimicrobial therapy is crucial for the outcome of these high risk patients. On the other hand, potential damages from adverse events and the contribution to the development of microbial resistance due to superfluous antimicrobial therapy must be duly considered. Meanwhile faster methods of microbiological identification, such as polymerase chain reaction,⁴⁹ mass spectrometry ionization (Matrix-Assisted Laser Desorption Ionization–Time of Flight), gene expression profiling, aptamers panels, etc.,⁵⁰ or even more sophisticated and individualized system-based ones (integrating genomics, metabolomics, and proteomics data), are not either widely available or fully developed,^{51–53} PCT dosing will remain one of the more useful tools to help clinicians decisions.^{5,54,55}

For these reasons, in the authors' opinion, it is worthwhile to use PCT measurements to have a more empowered prescription decision, even bearing in mind that the analysis of its levels does not fully ensure the desirable degree of precision.

The present work has manifestly some limitations that should be noticed. First, being a retrospective study it is more prone to selection bias than a prospective one. On the other hand, all patients enrolled came from the same center, so the results obtained may not be exactly reproduced in other Burn Units. Subgroup analysis according to associated pathologies was not done, neither the results from other current biomarkers like CRP or leucocyte counting were noted. However, according to the available literature, the relevance of these biomarkers is at least very questionable for the purposes of this study. Due to the small number of positive blood samples with fungi found during the study period, comparison with PCT levels in Gram-negative and Gram-positive sepsis was not done. To avoid confusion, mixed infections were purposely not included. It would also had been very interesting to further extend the analysis of PCT levels to the subsequent days after the positivation of blood samples, assessing the potential added value of PCT kinetics regarding distinction of different types of bacterial infection.

CONCLUSIONS

This retrospective study consistently showed the presence higher PCT levels in burn patients with Gram-negative sepsis, suggesting that PCT may help clinicians in the choice of the empirical antimicrobial therapy, while the definitive, gold standard, microbiological culture results and sensibility tests are not yet available. However, it should be emphasized that PCT must be integrated within the clinical context and the facility prevalent flora, and it can never substitute clinicians' evaluation and judgment. Prospective multicentric studies are needed to get a stronger validation of the use of PCT values for the distinction between Gram-negative and Gram-positive bacterial sepsis and it would be also desirable to include fungal and mixed infections. Evaluation of PCT kinetics potential for differential diagnosis between microbial sepsis due to diverse types of pathogens would also be very interesting and potentially useful for clinical practice.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Burn Care & Research* online.

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